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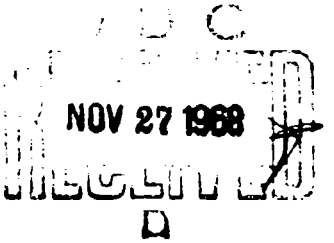


DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

CONTRIBUTION TO THE STUDY OF THE ANTIBIOTIC PROPERTIES OF CHLORPROMAZINE OR 4560 RP

[Followi- is a translation of an article by J. L. B -ion, of the Laboratoire de Bacteriologie de la Faculte de Medecine de Paris (Bacteriology Laboratory of the Medical School [University of] Paris, presented at the 6 July 1961 meeting of the Societe Francaise de Microbiologie (French Microbiology Society) and published in the French-language periodical Annales de l'Institut Pasteur (Annals of the Pasteur Institute), Vol 101, No 6, 1961, pages 876-886.]

The assumption of an action of chlorpromazine on lower organisms is not something recent. In fact, in 1953 Decourt and colleagues [2 and 3] [bracketed numerals refer to similarly numbered items in the bibliography appended at the end] made qualitative studies of the effect of increasing doses of chlorpromazine on the development of various germs, cultivated in plain water with the addition of peptone that had been seeded with 24-hour cultures. Moderate doses (approximately 30 µg per ml) inhibit the multiplication of the germs. Decourt compares this action with the action observed in higher organisms and speaks of "narcobiotic activity". This activity, almost universal, was observed with similar doses in such different microorganisms as bacteria, infusoria (Tetrahymena piriformis), seeds of higher plants (Lipidium sativum) or of lower mushrooms (Sterigmotocystis nigra). However, Decourt points out three exceptions to this rule: narcobiosis is observed in Escherichia coli, Salmonella lyphimurium and Acrobacter aerogenes only with chlorpromazine doses that approach 2,000 µg per ml. Decourt, finally, singles out a bactericidal power from this narcobiosis. The bactericidal doses are weak for some of the bacteria studied (50 µg per ml for a staphylococcus

or a streptococcus), extremely high for the rest (greater than 5,000 µg per ml for Escherichia coli or Clostridium septicum).

We have considered it interesting to review these facts in the light of techniques inspired by procedures currently recommended for studying fungal antibiotics.

MATERIAL AND TECHNIQUES

We used pure, powdered chlorpromazine (hydrochloride). The weighed product is put in aqueous solution. The desired dosages are obtained by means of successfive dilutions of the original solution. These solutions were always prepared extemporaneously.

1. During a first period, we made qualitative tests of the activity of chlorpromazine on eight germs, all of them aerobic:

Four gram-negative germs: Escherichia coli, Salmonella paratyphi A, Proteus vulgaris and Pseudomonas aerugi-nosa.

Four gram-positive germs: Staphylococcus aureus, Streptococcus fecalis, Bacteridium anthracis and Mycobacterium tuberculosis (H37Rv).

Increasing doses of chlorpromazine are put in stoppered tubes containing 10 ml water, with the addition of peptone, glucose and a pH indicator (this last addition, which facilitates reading, is not really useful except for weak doses of chlorpromazine, since strong doses acidify the medium). Inoculation is uniform: two drops of a culture in broth, 24-hours old. Two readings are taken, macroscopic and microscopic (fresh state, Gram) after twenty-four and forty-eight hours of incubation at 37°C.

For Mycobacterium tuberculosis we changed only the culture mediums and the reading times: the doses of chlorpromazine are put in Dubos' mediums with Tween-80, inoculated with five drops of a culture of Mycobacterium tuberculosis in Dubos' medium with Tween-80, eight days old. Readings were taken after eight and fifteen days incubation at 37°C.

2. In a second period, since we had an idea of the inhibitory doses of chlorpromazine, we attempted to ascertain

whether the observed action was bacteriostatic or bactericidal. We repeated the preceding experiments, but, instead of being satisfied with an approximate evaluation, we counted the germs present in our water containing peptone and glucose plus chlorpromazine, as well as in control water containing peptone and glucose. We made our count at the moment of inoculation, after twenty-four hours and after forty-eight hours of incubation at 37°C., always using the same procedure: removal of a drop of the liquid medium being studied. Successive dilutions in 1:10, 1:100 1:1000 and 1:10000 physiological water. A streak is made with a loop on agar poured the evening before into a Petri dish, for each of the dilutions as well as for the non-diluted medium. The count is made after incubation for twenty-four hours at 37°C. The figures correspond to the number of colonies located on the streak that carries the most frequently readable dilution.

3. Next, while studying an eventual resistance that was acquired to chlorpromazine, we were able to perform only one experiment on a liquid medium for only four germs.

For Staphylococcus aureus and Streptococcus fecalis, we inoculated a series of tubes, containing 10 ml to which peptone, glucose and a pH indicator had been added, plus increasing doses of chlorpromazine ranging from 10 to 300 µg per ml, with two drops of a 24-hour old broth of the germ being studied. After incubation for twenty-four hours at 37°C., the tube corresponding to the highest dose of chlorpromazine that still permitted cultivation is removed and its contents will be used to seed (two drops per tube) a new series of water containing peptone and glucose plus chlorpromazine, as well as a control tube. Sixteen successive passages are performed.

The preceding technique cannot be used for Proteus vulgaris and Pseudomonas aeruginosa, due to the large amounts of chlorpromazine that are required. The experiments, still based on the same principle, were performed in hemolysis tubes containing 1 ml water with peptone and glucose plus chlorpromazine. Inoculation was with one drop of a 24-hour old broth, diluted to 1:100.

4. Finally, we studied, in viva, an eventual activity that was both preventive and therapeutic.

We inoculated subcutaneously three lots of fifteen white mice with a virulent strain of Diplococcus pneumoniae:

the first lot, with 0.5 ml of a 24-hour old culture in ascitic bouillon; the second one, 0.5 ml of the same culture diluted to 1:10000; the third one, with 0.5 ml of a 1:10,000,000 dilution. Each lot was divided into three series; the first series served as control; the second one had already received, the evening before, a subcutaneous injection of 20 mg per kg of chlorpromazine; this injection was repeated at the time of the microbian inoculation. The third series received, simultaneously with the germ, 20 mg per kg of chlorpromazine, both administered subcutaneously in different points. The survivors of series 2 and 3 received a subcutaneous injection of 20 mg per kg of chlorpromazine every twenty-four hours.

The same experiment was performed on two lots of mice that were inoculated with 0.5 ml of a 24-hour old culture of Bacteridium anthracis, pure and diluted to 1:50, and on a batch of mice inoculated with a pure culture of Staphylococcus aureus. All the dead animals were autopsied and verification seedings were made. Organ smears (liver and spleen) were made systematically and were stained by means of Gram's method.

RESULTS

1. First Series of Experiments. It reveals an undeniable ability to inhibit microbian development (Cf. Table I).

Table 1

Germes étudiés	Doses de chlorpromazine (en µg/ml)															Témoin
a	1	10	20	30	40	50	100			400	500	1 000	2 000	3 000	4 000	c
Escherichia coli	+ G	+ G	+ G	+ G	+ G	+ G	+ G	−	−	−	−	−	−	−	−	− G d
Salmonella paratyphi A	+ G	− G	− G	− G	− G	+ G	+ G	−	−	−	−	−	−	−	−	− G
Proteus vulgaris	+	+	+	+	+	+	+	+	− (8 h)	−	−	−	−	−	−	+ Gram négatif
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	− (8 h)	+ (8 h)	−	−	−	−	−	+
Staphylococcus aureus	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+ e
Streptococcus fecalis	−	− (8 h)	−	−	−	−	−	−	−	−	−	−	−	−	−	+ Gram positif
Bacteridium anthracis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Mycobacterium tuberculosis	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+

(Legend on next page)

/Legend:/ a) Germs studied; b) Doses of Chlorpromazine (in μg per ml); c) Control; d) Gram-negative; e) Gram-positive.

Although the gram-negative germs are only sensitive to relatively high doses (100 μg or more), the gram-positive germs that were studied are inhibited by weak doses (a few μg). Mycobacterium tuberculosis does not escape from this rule. (However, according to subsequent experiments, it seems to us that this germ may easily and rapidly acquire a resistance, which, moreover, is relative).

In the course of these experiments, we were able to study the morphology of the germs subjected to the action of chlorpromazine. All the motile germs that we studied preserve their motility in the presence of chlorpromazine.

With Gram Staining, the alterations are more or less considerable: Bacterium anthracis and Streptococcus fecalis display only a relative irregularity in size. Staphylococcus aureus is much more interesting. Masses of enormous levuriform shapes, with slightly blurred edges, weakly gram-positive (all the more weak as the components are larger), resembling protoplasts, appear in the middle of unaltered fields. We were able to observe identical shapes on the same germ cultivated in a hypertonic medium plus chlorpromazine. We found the same levuriform shapes with a spheroplastic appearance (gram-negative this time) on the gram-negative germs that we studied. Moreover, greater modifications of tinctorial affinities (certain elements of normal shape retain the Gram stain quite definitely) and a clear tendency toward auto-agglutination must be noted. All these facts can do nothing more than suggest a considerable change in the microbian wall.

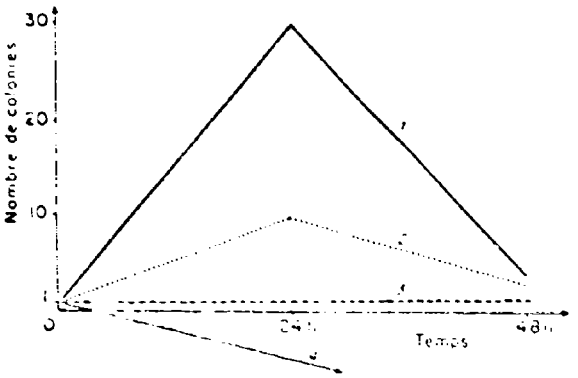


Fig. 1. Growth curves of Staphylococcus aureus in the presence of chlorpromazine (number of colonies in one drop of a 1:1000 dilution of the medium being studied). 1. Control curve; 2. In the presence of 1 μg per ml of chlorpromazine; 3. In the presence of 10 μg per ml of chlorpromazine; 4. In the presence of more than 120 μg per ml of chlorpromazine.

[Legend:] Nombre de colonies = number of colonies; temps = time.

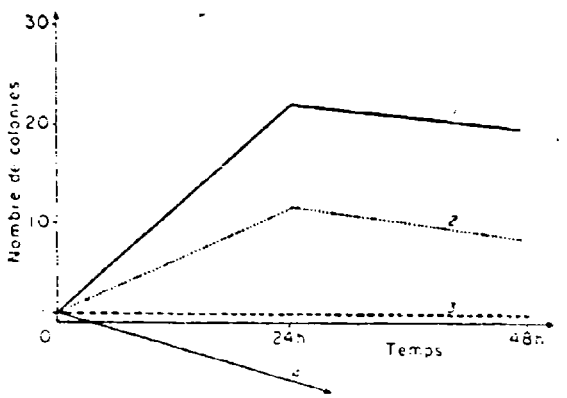


Fig. 2. Growth curves of Bacteridium anthracis in the presence of chlorpromazine (number of colonies in 1 drop of a 1:100 dilution): 1. Control curve; 2. In the presence of 2 μg per ml of chlorpromazine; 3. In the presence of 4 μg per ml of

chlorpromazine; 4. In the presence of more than 5 µg per ml of chlorpromazine. (Same legend as for Fig. 1).

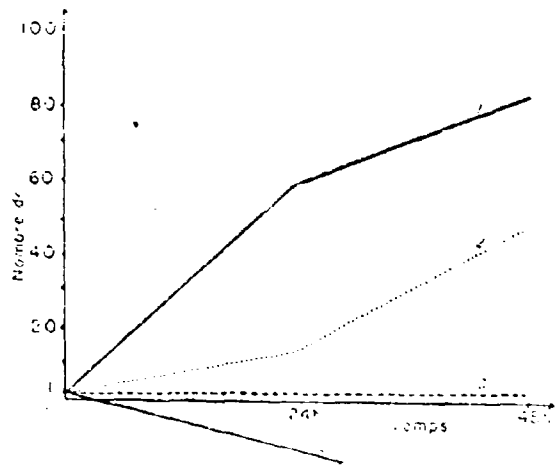


Fig. 3. Growth curves of Salmonella paratyphi A in the presence of chlorpromazine (number of colonies in 1 drop of a 1:10000 dilution): 1. Control curve. 2. In the presence of 10 µg per ml of chlorpromazine. 3. In the presence of 200 µg per ml of chlorpromazine. 4. In the presence of more than 250 µg per ml of chlorpromazine. (Same legend as for Fig. 1).

2. Second Series of Experiments. A complete report of all the counts made would be dull, therefore we shall only give the figures for the three dosages of chlorpromazine that have an activity of different nature, still referring to the counts made of a control culture. Of course, all the intermediary stages exist between the apparent inactivity of the product and the three activities taken as typical.

We present these results (Figs. 1, 2, 3 and 4) in the form of microbian growth curves.

Regardless of the germ under consideration, the action on the chlorpromazine always seems to be the same: with weak

doses, a bacteriostatic action that keeps increasing with the chlorpromazine doses, terminating finally with a total inhibition of the growth. With still stronger doses, the action becomes bactericidal. Thus, the inhibitory power of chlorpromazine on microbian development must be compared with the inhibitory ability of an antibiotic.

Figure 4 shows us, on the other hand, that Pseudomonas aeruginosa may very rapidly become resistant to doses of chlorpromazine that are initially bactericidal.

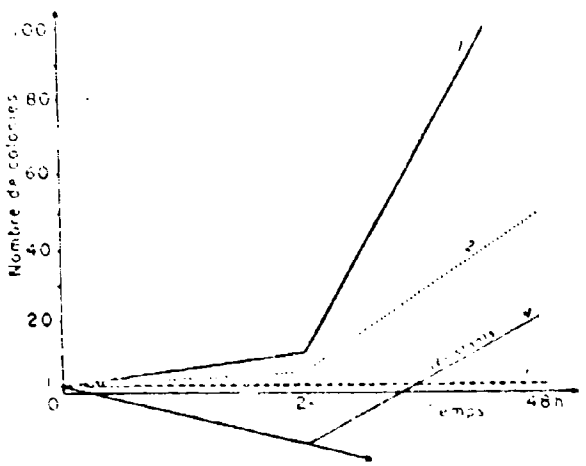


Fig. 4. Growth curves of Pseudomonas aeruginosa in the presence of chlorpromazine (number of colonies in 1 drop of a 1:10000 dilution): 1. Control curve. 2. In the presence of 200 µg per ml of chlorpromazine. 3. In the presence of 400 µg per ml of chlorpromazine. 4. In the presence of 500 µg per ml of chlorpromazine.

[Legend:] resistants = resistant (otherwise same as Fig. 1).

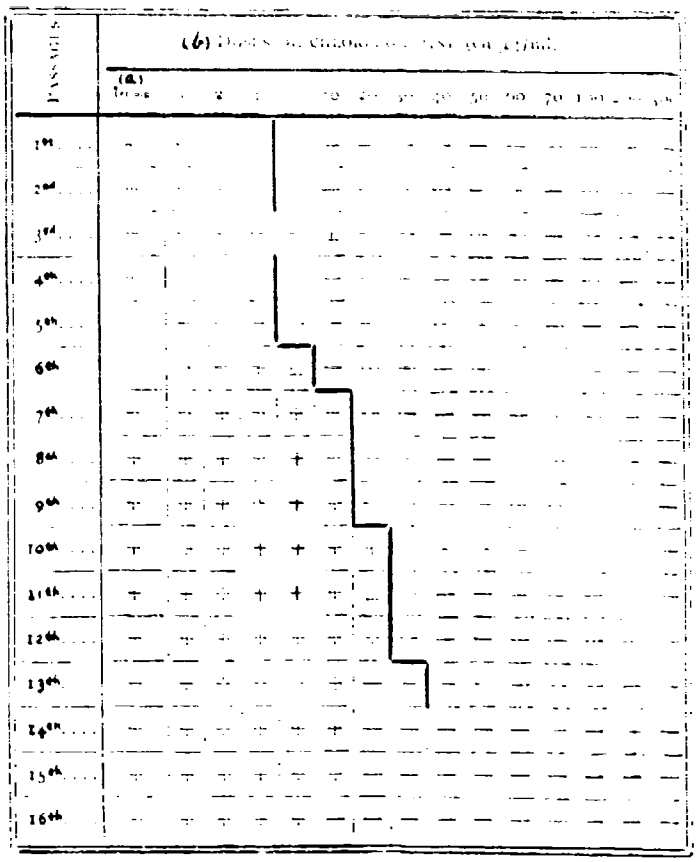
3. Study of the Acquired Resistance. We found an acquired resistance of a penicillin type in three of the four germs studied, with the germs adapting themselves in multiple stages (Cf. Tables II, III and IV).

The germs that have become resistant may lose their resistance secondarily, with the decrease still occurring in successive steps.

The resistant germs always seem to us to have a normal morphology.

Table II

Study of the Resistance to Chlorpromazine Acquired by a Strain of Staphylococcus aureus



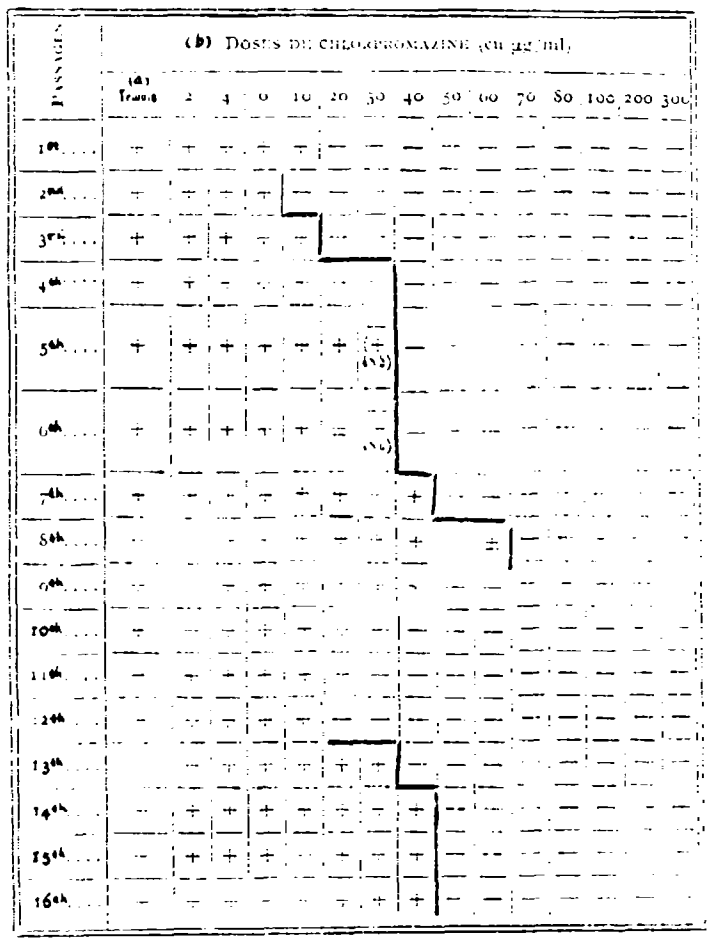
/Legend:/ a) Control; b) Doses of chlorpromazine (in μg per ml).

4. Study in vivo. Although chlorpromazine had an undeniable narcoleptic action on the inoculated mice, it showed itself, unfortunately, to be lacking in any anti-infectious power, either preventive or therapeutic. All

the treated animals died in the same period of time as the control animals and presented the same visceral lesions.

Table III

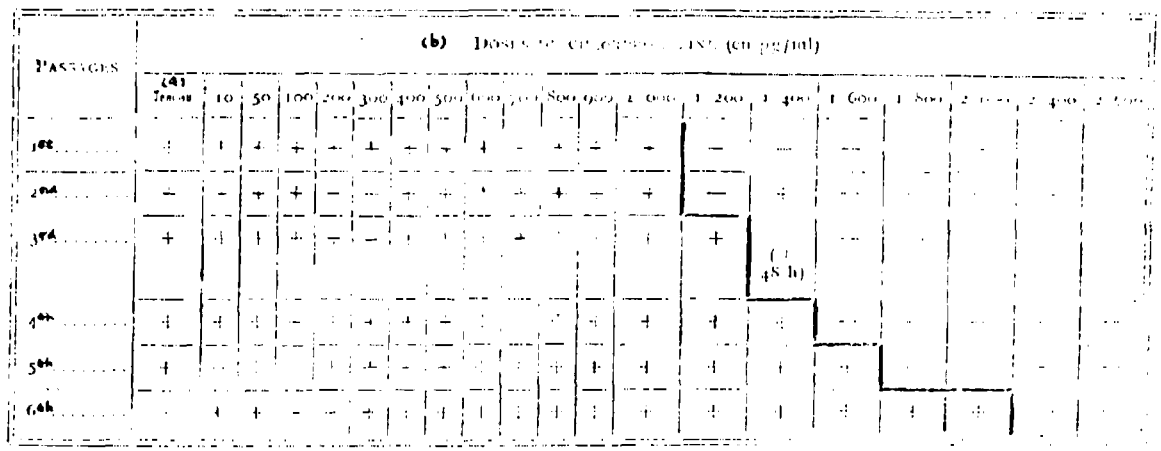
Study of the Resistance to Chlorpromazine Acquired by a Strain of Streptococcus fecalis



/Legend:/ a) Control; b) Doses of chlorpromazine (in μg per ml).

Table IV

Study of the Resistance to Chlorpromazine Acquired by a Strain of Pseudomonas aeruginosa



/Legend:/ a) Control; b) Doses of Chlorpromazine (in μg per ml).

DISCUSSION

Several orders of facts seem to stand out: chlorpromazine acts on germs like an antibiotic. This activity does not appear to be exerted in the same degree on all of them: although 5 µg per ml are bactericidal for Staphylococcus aureus, more than 500 are necessary to obtain the same action on Pseudomonas aeruginosa. In spite of the small number of species studied, it seems indeed that chlorpromazine involves primarily gram-positive germs, since gram-negative germs have a quite considerable natural resistance. Thus chlorpromazine probably acts like a narrow bacterial spectrum antibiotic. A comparison with penicillin developed which was to be strengthened, on the one hand, by a resistance acquired in multiple steps, on the other hand by changes in the microbian wall. Therefore, if we had to describe the antibacterial action of chlorpromazine in one word, we would speak of an antibiotic of a penicillin type. This manner of acting, which appears to be specifically microbian, seems to us to be scarcely comparable with the activities of chlorpromazine on higher organisms. Chlorpromazine seems to us to be a neuroleptic and an antibiotic. We do not believe that it is a "narcobiotic", to repeat Decourt's word. Only the exact knowledge of the mechanisms of action of this drug could solve the problem definitively, but we are still quite far from it.

SUMMARY

In vitro, chlorpromazine behaves like a narrow bacterial spectrum antibiotic limited to gram-positive germs. It is responsible for parietal alterations. With respect to it, germs may acquire a resistance in multiple steps.

In vivo, chlorpromazine does not appear to have any action, either preventive or therapeutic.

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